EFFICACY REVIEW

PRODUCT:	89670-R, Black Pearl Paste Lodi Group. Grand Fougeray, France
DATE:	August 10, 2017
DP NUMBER:	441856
DECISION NUMBER:	474788
GLP:	No
CHEMICAL:	Alphachloralose
EPA PC CODE:	476200
PURPOSE:	Review submitted field efficacy data to determine if they support registration of the new active ingredient alphachloralose.
MRID:	50273801
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BACKGROUND:

Since early 2013, Lodi Group of Grand Fougeray, France has pursued registration of the new active ingredient alphachloralose. Two products are proposed for registration, including a technical product (89670-E) and an end-use product (89670-R) containing 4.0 or 4.45% alphachloralose.[1] As alphachloralose is not currently registered in the U.S., Lodi is in the midst of submitting the EPA-required data for a new rodenticide active ingredient for use against house mice (*Mus musculus*) indoors.

The efficacy studies required to support U.S. registration for a new active ingredient rodenticide for use against house mice are listed below.

1. A study that establishes the acute oral LD_{50} of the chemical for house mice.
2. A laboratory study that assesses the palatability and lethality of a bait containing the chemical against wild-type house mice (*Mus musculus*).
3. Five indoor field trials, each conducted in a different region of the U.S.
4. One outdoor field trial (if no claims for controlling house mice via outdoor placements is proposed, this requirement does not apply).

[1] As was noted by Bill Jacobs in a previous efficacy review dated 04/26/16, the proposed CSF and label describe different nominal amounts of active ingredient for some reason.

Lodi's U.S. agents have previously expressed difficulty in getting entities to perform these tests and have proposed to reduce the number of indoor regional field trials from 5 to 3, a number upon which EPA has agreed. Thus far, Lodi has fulfilled the acute oral LD_{50} requirement, as well as 1 of the 3 indoor regional field trials.[2] The data reviewed below were submitted to address the 2nd of the proposed indoor regional field trials.

DATA SUMMARY

Buczkowski, G. (2016) Field Evaluation of Black Pearl Paste (Alphachloralose) against the House Mouse, *Mus musculus*, in a commercial Equine Facility. Unpublished study prepared by Summit Research and Consulting, LLC. 63p.

MRID# 50273801

This study describes a field trial conducted at "Tanglewood Stables, a privately owned horse farm located in Clemmons, North Carolina". Buczkowski describes the site as having "a moderate house mouse (*Mus musculus*) infestation", with no rodenticide applications having occurred in the 10-15 years preceding this trial. According to the report, "mice [were] concentrated mainly around watering buckets and areas where hay and bagged horse feed are stored". Structures at the site which were selected for treatment included

> three stables of equal size (40 ft wide by 85 ft long). Stables 1 and 2 were single level and stable 3 consisted of two levels. Level 1 of stable 3 included all the horse stables and Level 2 was an open space room used for hay storage.

Like the Buczkowski Indiana field trial reviewed by Bill Jacobs in an efficacy review dated 04/26/16, house mouse activity before and after baiting was to be determined using census baiting, tracking scores, and live-trapping. A trap-out phase reportedly occurred following the post-treatment census period.[3] Inexplicably, the specific dates and times that these methods occurred are not provided in the raw data submitted with this report. Nevertheless, the sequence of events, at least to the extent that can be determined, is provided below.

04/21/16 – 04/26/16: Pre-treatment census period (census bait consumption, tracking scores, and live trapping)
Pre-treatment lag period: 4 days
05/01/16 – 05/11/16: Toxic bait exposure period (bait take measured on days 1, 2, 5, 7, and 9)
Post-treatment lag period: 4 days
05/16/16 – 05/21/16: Post-treatment census period (census bait consumption, tracking scores, and live trapping)
05/23/16 – 05/30/16: Post-treatment snap trapping

For the pre-treatment census baiting, Detex Blocks (20-g rodenticide-free bait blocks produced by Bell Laboratories, Inc.) were placed in 48 "Protecta" mouse bait stations. Two Detex Blocks were reportedly placed in each bait station. After 24 hours, the amount of census bait remaining in each station was determined. A second, 24-hour census bait removal period, 2 days after the first, was utilized during the pre-treatment census period and then again during the post-treatment census period. In this way, census bait removal occurred 2 times during the pre- and post-treatment census periods.

Tracking activity was performed using "6 by 6 inch PVC floor tiles coated with blue construction chalk (25%, mixed in isopropyl alcohol)". Thirty-six (36) total tracking tiles were used, with 12 having been placed

[2] Data submitted to address the laboratory palatability/lethality criterion were submitted concurrently with this field trial, and have been reviewed in a separate efficacy review dated 08/02/17, DP #441854.
[3] Trap-outs or "snap trapping" is not appropriate as a true activity index as the traps themselves result in a permanent removal of individuals from the population. Instead, snap trapping is employed at the end of rodenticide field trials to identify whether any residual activity is occurring from the target rodents (or in some cases, whether other species may have been "masquerading" as house mice, inflating census or toxic bait removal figures during the trial).

"approximately 3 feet away from the bait stations" in stables 1 and 2, and 24 placed in stable 3 (12 on level 1, and 12 on level 2).[4]

Marks on tracking patches were scored using a 1-inch grid which was placed over each 6 x 6" tile, creating 36 individual squares which were counted as marked or unmarked. The number of "marked squares" was then divided by 36 to convert it into a percent, which was rounded to the nearest whole number. Tracking scores were calculated 24 hours after the tiles were initially placed. Similar to the census baiting, a second, 24-hour tracking period, two days after the first, was utilized during the pre-treatment census period and then again during the post-treatment census period.

Live trapping was performed with the use of multiple catch traps (JT Eaton 420CL), in areas along walls with "high mouse activity". Four (4) traps were used in each of stables 1 and 2, and 8 were used in stable 3 (4 on level 1, and 4 on level 2). Traps were reportedly placed for 12 hours, spanning the time from when they were set (8 PM) and checked the following morning (8 AM). Like the other 2 census methods, live trapping was apparently repeated a 2nd time, possibly 12 to 24 hours following the first census period.[5]

For the 10-day toxic baiting period, 48 bait stations were "placed along the walls in horse stalls, feed rooms, tack rooms, and other [sic] around areas of high mouse activity as revealed during the preliminary inspections". Buczkowski reports that

> A total of 1,555 grams of Black Peral bait were put out across all 3 stables. During the toxic baiting period, the mice consumed a total of 1,172 grams of bait, equivalent to 75%.

and then

> [the] bait appeared attractive and effective in small amounts, despite the presence of competing food. Periodic inspections of the bait stations throughout the toxic baiting period (days 1, 2, 5, 7, and 9) revealed that most of the bait was consumed during the first 5 days. The first symptomatic mice were observed within 1 hour after the baits were deployed and virtually all dead mice were found on days 1, 2, and 5.

These results differed somewhat from those reported by Buczkowski for the Indiana field trial, where it was reported that "the first symptomatic mice were observed 8 hours after the baits were deployed", and that "virtually all of the dead mice were found on days 1 and 2" (Buczkowski, 2015). In any case, these results and those from the Indiana field trial suggest that the bait affects the mice which have consumed a sufficient amount of it rather quickly.

The post-treatment censuses occurred in an essentially identical manner as for the pre-treatment censuses, with pre- and post-treatment figures bracketing the 10-day toxic baiting period. Post-treatment snap trapping occurred at the end of the trial (05/23/16 – 05/30/16), with 8 "victor easy set traps" used in each of stables 1 and 2, and 16 used in stable 3 (for a total of 32 traps). As snap trapping was carried out for 7 nights, there were 224 "trapnights" possible. Snap traps were reportedly "checked for mice after 24 hours and re-set as necessary. The trap-out continued for 7 days and the total number of mice caught was recorded". While capturing residual individuals of the target species in question (house mice) is the most obvious purpose for post-treatment snap

[4] It is unclear whether and to what degree the tracking tile placement locations were independent from the census bait placement locations.

[5] As no raw data forms were provided in the report, it is not clear exactly when the 2 rounds of live trapping occurred pre- and post-treatment. For live trapping to have been truly independent, it should not have occurred in such a way as to overlap either of the other 2 census methods.

trapping, any "tripped" traps (i.e., traps sprung but with no capture) and/or the capture of non-targets should have also been reported, if they occurred.[6]

Results reported for pre- and post-treatment census bait take are provided in the tables below.

Pre-treatment census	
Period	**Total census bait take (grams)**
1st	1274.2
2nd	1353.1
total	2627.3

Post-treatment census	
Period	**Total census bait take (grams)**
1st	53.5
2nd	122.0
total	175.5

Census bait take was slightly higher on the 2nd pre-treatment census day (1353.1 grams) compared to the 1st (1274.2 grams). A small amount of post-treatment census bait removal occurred during the 1st post-treatment census (53.5 grams), with an amount more than double that having been removed the following census day (122.0 grams). Whether this post-treatment census bait removal was from "new" mice backfilling the site or from some number of "resident" mice which were rendered bait-shy is unclear.

As toxic bait consumption was reported in a somewhat unusual manner in the report[7], it is difficult to determine the amount of bait removed per station, per day. What is most important for the purposes of this review is that a total of 1,172 grams of toxic bait were reportedly removed during the 10-day toxic baiting period, with much of it having been removed very early during that period.[8]

Maximum pre- and post-treatment tracking scores, census bait removal, and the number of house mice captured during live trapping are provided in the table below.

Activity index	Pre-treatment	Post-treatment	Percent change
Census baiting	1353.1 grams	122.0 grams	91.0%
Tracking scores	1496	21	98.6%
Live trapping	19	1	94.8%

Estimates of activity reduction in house mice were >90% by all 3 census methods, and exceeded EPA's criterion of a minimum 70% reduction in activity via 2 independent census methods for field efficacy trials of lethal rodenticides. These results suggest very good, if not complete, control of a relatively large number of house mice.[9]

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[6] Generally, in the event of "tripped" traps with no capture, some adjustment is made to the number of trapnights (e.g., counting a tripped trap as ½ of a trapnight) to account for traps which were no longer able to capture the target animals due to them being temporarily out of commission.

[7] Rather than reporting how much of the toxic bait was removed per station per day, Buczkowki reported "bait added back to the station" per station per day.

[8] This is a pretty typical result for baiting with acute rodenticide baits.

[9] A crude estimation of house mouse numbers present during rodenticide field trials is to count every 2.5 grams of "grain" removed on a given day as being equal to about 1 mouse. While removal of grain by house mice probably differs somewhat from how bait block material is removed by the same species, a ballpark figure of several hundred house mice present at the sites is a reasonable estimate.

Two (2) house mice were reported as captured during the post-treatment snap trapping, providing a trapnight index (TNI) of about 0.009. As no raw data entries related to snap trapping were presented in the report, it cannot be determined whether any traps were tripped with no capture, or whether any non-targets were captured. Nevertheless, this reported figure is consistent with the reductions reported for the census methods and falls well below the "level of concern" for post-treatment snap trapping (i.e., a TNI $\leq$ 0.1).

It is unclear whether the carcass searching described in the report was performed using standard procedures for these types of trials (i.e., marking and walking a transect of a predetermined distance and for a set period of time). The report indicates that

> a mouse recovery experiment was performed during [the post-treatment census period] to determine if any of the dead or symptomatic mice discovered in the bards recovered from the effects of the rodenticide. The mice were collected and kept in areas where initially discovered to assure that the temperature during recovery was the same as the temperature during feeding and poisoning. To prevent the mice from escaping (if recovered), a wire mesh pen holder (6 in tall by 3 in diameter) was placed over each mouse (one mouse per cup). A brick was placed on top of the inverted cup to prevent removal by non-target animals or farm workers. The condition of the mouse was evaluated 24 h later and noted as either dead or alive. Four mice were collected in each of the three stables and individually evaluated for possibly recovery (12 mice total).

Of the 12 mice which were recovered and retained per the procedure just described, all were dead when inspected the following day (24 hours later). According to the report, "death was evident as the mice had stiffened (rigor mortis), their eyes turned white, and some had begun to decompose and attract flies". This confirmatory procedure is helpful to consider with the reported results, as alphachloralse has a long history of use as an anesthetic. Clearly the users of this product would be interested in killing mice rather than temporarily putting them to sleep.

An analysis of the test bait was appended to the back of the report, with a bait manufacture date of 01/12/2015. Thus, the test bait was over a year old at the time it was deployed for the toxic baiting period in this trial. An analysis for percent active ingredient is indicated to have occurred on 01/13/15 (the day after the bait was created), with a reported result of 4.27% alphachloralose having been present. The other ingredients provided on this same form are consistent with the CSF currently proposed for registration.

CONCLUSIONS

Taken at face value, this field trial describes a very successful removal of house mice from a commercial equine facility in North Carolina (i.e., the Southeast region of the U.S.). This field trial is accepted in support of registration, despite the problems previously mentioned within this review.

References

Buczkowski, G. 2015. Field evaluation of Black Pearl Paste (alphachloralose) against the house mouse *Mus musculus*, in a confined livestock facility. Unpublished report, Summit Research and Consulting, West Lafayette, IN, 97 pp.